

formed due the haemolysis and CD163, whereby fewer Hp-Hb complexes are taken up by the macrophages leading to a less severe hemosiderosis.

5 In another embodiment the same effect may be obtained by administrating antibodies directed to the CD163 receptor. The antibodies may be monoclonal, such as those mentioned below in the examples or polyclonal. Production of antibodies is known to the skilled person.

10 In a further embodiment Hp-Hb complexes are administered to inhibit uptake of native Hp-Hb complexes again leading to a less severe hemosiderosis.

15 In yet a further aspect of the invention the CD163 variant is used in a method for the removal of at least one Hp-Hb complex in serum and/or plasma of an individual. Since the present inventors have now established CD163 and CD163 variants as the acute phase-regulated capture protein for Hp-Hb complexes the CD163 variant may be applied to an individual in need of plasma haemoglobin clearance.

20 This may also be accomplished by gene therapy, by administration of genes encoding CD163 or a variant thereof, in order to produce cells capable of assisting the macrophages in case of plasma haemoglobin clearance.

25 In another embodiment of the invention the CD 163 variant is used in a diagnostic method. One such diagnostic method is for marking a cell expressing a CD163 variant, wherein at least one of the haemoglobin or haptoglobin molecules or parts thereof are labelled. It is possible to identify CD163 variants *in vitro* as well as *in vivo* by bringing into contact at least one Hp-Hb complex with an environment comprising CD163 variants. The individual haemoglobin or haptoglobin molecules may be labelled with a marker as discussed above. In one aspect of the invention the CD163 variant is used in a diagnostic method for identifying monocytes and/or macrophages in an individual or *in vitro*.

30 In another aspect the CD163 variant is used in a method for the identification of at least one Hp-Hb complex in serum and/or plasma of an individual.

35 In this aspect the CD163 variant may be used for determination of the haemolysis rate of an individual.

Furthermore, the Hp-Hb complex linked to a marker may be used for identification of monocytes, such as macrophages, in tissues, such as sections of tissues for example for microscopic examinations.

In another embodiment the Hp-Hb complex linked to a marker may be used for detection of CD163, either membrane bound CD163 and/or soluble CD 163. In particular the Hp-Hb complex linked to a marker may be used for detection soluble CD 163 in a sample, such as a blood sample. This could also be detection using labelled Hp-Hb complex. The label could be a chromophore, a fluorochrome, a radioactive isotope, biotin or an enzyme

The invention also relates to the following applications of detection of soluble CD163. CD163 may be detected by any of the methods described above in relation to Hp-Hb complex. Furthermore CD163 may be detected by any other method known to the person skilled in the art, such as through the use of antibodies, monoclonal and/or polyclonal, directed to CD163. This could also be detection using labelled antibodies. The label could be a chromophore, a fluorochrome, a radioactive isotope, biotin or an enzyme.

Furthermore, CD163 may be detected using labelled Hemoglobin (Hb) and/or haptoglobin, labelled as discussed above for antibodies.

The detection of soluble CD163 may be used as tools in diagnosis, monitoring and control of patients.

For example, one use of soluble CD163 is as a diagnostic marker in diagnosis, monitoring, and control of patients with hemolysis and/or other hematological conditions (e.g. aplastic anemia, iron-deficiency anemia, megaloblastic anemia, sickle-cell anemia, polycythemia, malaria, leucemia, myelodysplasia, lymphoma, leukopenia, splenectomy).

Another use of CD163 is as an acute phase marker, because soluble CD163 is upregulated during acute phase response. Hence Soluble CD163 can be used in diagnosis, monitoring, and control of patients with inflammation (infection, cancer, autoimmunity) as well as in diagnosis, monitoring, and control of patients with immunodeficiency.

Still another use is in monitoring, and control of patients treated with glucocorticoids and/or cytostatics and/or other medications.

The concentration of soluble CD163 may be determined using any suitable methods. One of the following techniques are particularly suitable:.

One assay could be Sandwich-ELISA and/or competitive-ELISA using a detection system, which could be peroxidase-labeled antibody/OPD system, other enzymes than peroxidase, chemiluminescence, fluorescence, biotin-avidin-systems.

Another assay could be nefelometric- or turbidimetric assays, radio-immuno-assays (RIA), purification of CD163 by e.g. chromatography or electrophoresis and detection by e.g. photometry, chromatography combined with mass-spectrophotometry.

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The CD163 concentration could be determined in serum and plasma, which could be stabilised with EDTA, citrate or heparin, as well as in blood, urine, cerebrospinal fluid, and other body-fluids of human and/or animal origin. Furthermore the assays can be used for measuring the concentration of CD163 in artificial media e.g. cell-culture-media.

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### Experimentals

#### Example 1

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#### Purification and identification of the Hp-Hb receptor.

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Human Hp (1-1, 2-2, and mixed phenotypes) and human Hb (A<sub>0</sub>, A<sub>2</sub> and S forms) were from Sigma. A five ml Hp-Hb Sepharose CL-4B (Pharmacia-Amersham) column was prepared by coupling complexes of Hp (5 mg, mixed phenotypes) and Hb (4 mg, type A<sub>0</sub>). The column was loaded with 100 ml ~1% Triton X-100 solubilised membranes (from human spleen, placenta, and liver), prepared as previously described (Moestrup, S. K., Kalløft, K., Sottrup-Jensen, L. & Gliemann, J. The human  $\alpha_2$ -macroglobulin receptor contains high affinity calcium binding sites important for receptor conformation and ligand recognition. *J. Biol. Chem.* **265**, 12623-12628 (1990)). The purified 130 kDa protein binding Hp-Hb was eluted in 10 mM NaH<sub>2</sub>PO<sub>4</sub> (pH 6), 150 mM NaCl, 5 mM EDTA and 0.5% CHAPS (Aldrich). SDS-gel separated protein was processed for tryptic digestion and MALDI mass spectrometry by Protana (Odense, Denmark). The difference in calculated and measured masses was for all peptides less than 0.042 kDa. The murine monoclonal CD163 antibodies EDHu-1 (Serotec) and GH1/61 (Research Diagnostics) were used for western blotting. A polyclonal CD163 antibody was raised by immunisation of a rabbit with ligand-affinity purified receptor.

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#### Example 2

#### Ligand-receptor binding analysis